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HYPOLEUCOCYTOSIS

BY

JAMES EWING, M. D.

TUTOR IN HISTOLOGY, COLLEGE OF PHYSICIANS AND SURGEONS;
ASSISTANT PATHOLOGIST TO ROOSEVELT HOSPITAL, NEW YORK

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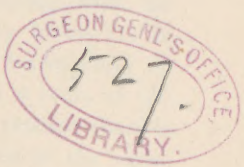

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TOXIC HYPOLEUCOCYTOSIS.*

THE phenomenon which has attracted most attention in the pathology of the blood in recent years is that of inflammatory leucocytosis. That this delicate element of a fluid tissue, the leucocyte, should be able to muster within a few hours in quadruple or even sextuple numbers in the blood, is truly a matter to excite admiration or even incredulity.

It is the part that leucocytosis may play in the production of immunity which lends to the subject to-day one of its chief interests. That large numbers of these cells showed a tendency to surround all sorts of foreign bodies in their vicinity was a fact eagerly seized upon by adherents of the cellular theory of immunity, and soon became for them a chief factor in argument.

Naturally, investigation was first directed to the most pronounced example of this condition offered by disease, so that to-day more is known about leucocytosis in pneumonia than in any other malady. Beginning with the interpretation of the *crusta phlogistica* by Piorry (1) in 1839, and the first formulated theory of leucocytosis by Virchow (2) in 1871, in the last ten years a score of observers have been over the ground, and at present there is comparative unanimity in the interpretation of the variations of leucocytosis in pneumonia.

But the ready assumption of the phagocytic nature of leucocytosis by adherents of the cellular theory

* During the course of this work the writer has greatly profited by the many timely suggestions of Professor T. Mitchell Prudden.

of immunity, and the evident importance of the phenomenon in the course and limitation of disease, elicit yearly new and varied theories of the origin and nature of leucocytosis.

Virchow's theory of simple proliferation first appeared. He presumed that a new formation of leucocytes occurred through simple proliferation in the lymph nodes, in the swollen bronchial nodes of pneumonia, in the hypertrophied chains of leucæmia, and through the general lymphadenitis of syphilis. He failed to explain, among other things, the failure of leucocytosis in pseudo-leucæmia, in typhoid fever, and in tuberculosis.

Roemer (3) attributes leucocytosis to the action of products of tissue metabolism and bacterial life, especially to alkali-protein. These products, he believes, reach the blood and lymph from their point of origin in the tissues, and exert a direct chemotactic and formative influence on the leucocytes. The formative influence acts in the circulating blood, and produces an increased number of leucocytes by the method of amitosis. By chemotaxis the new leucocytes are drawn into general or local circulation. That chemotaxis plays a part in the phenomena of leucocytosis is generally admitted, but that amitosis has been demonstrated as the method of origin of polynuclear leucocytes is denied by most histologists (Rieder (4)). Roemer's chemotactic theory stands, therefore, neither proved nor disproved.

Vom Limbeck's (5) theory of "exudation" is in partial agreement with Roemer's. He believes that chemical bacterial products cause a reaction on the part of the tissues, with exudation and leucocytosis. This reaction, which is to be regarded as Nature's effort at healing, is specially directed to the spleen, lymph nodes, and marrow, from which he holds the new leucocytes to be principally derived. Thus there are more leucocytes in the splenic vein than in the veins of the abdominal wall. Von Limbeck failed to prove that there were more leucocytes in the splenic vein than in other visceral veins; the relation between exudation and leucocytosis is not constant, and his theory does not explain hypoleucocytosis.

Since 1872 it has been known that the injection of

curari into the blood caused a disappearance of leucocytes. Similar results were noted later after the injection of fibrin ferment, pus, peptone, etc. Finally, the same phenomenon was observed after injection of bacteria and their products (6). The great constancy with which the diminution precedes the increase of leucocytes impressed Lowit (6) with the idea of the essential character of this sequence, and led him to find in the impoverishment of the blood the cause of leucocytosis. The exact manner of the disappearance of the leucocytes he held to be an actual destruction or solution (leucocytolysis). The regeneration of the blood in hyperleucocytosis he referred to an increased activity of the blood-producing organs, the lymphocyte being the initial form in which the new cells reach the blood. Neither mitosis nor amitosis occurred with sufficient frequency in the circulating blood to account for the abundance of new leucocytes. During the stage of hypoleucocytosis Lowit proved that the diminution of leucocytes is general over the superficial vessels. He states that he found this diminution to prevail also in the central vessels, but the extent of his observations on this latter point he does not state. The numbers of leucocytes actually found in the central vessels, any comparisons he may have made with the normal numbers existing there, and the conditions under which he drew the specimens of blood, he does not report; but admits having found great variations in the condition of the central vessels. Schulz (7), therefore, finds in Lowit's statements no proof that the leucocytes are not increased in the central vessels while diminished at the surface. Moreover, Lowit fails to explain sufficiently why the new cells to be found in leucocytosis are multinuclear, while maintaining that the form in which they are produced by the organs is uninuclear. Finally, at no stage after the intravenous injection of bacteria does he find in the blood sufficient evidence of recent destruction of leucocytes.

Bieganski (8) has recently offered some new suggestions as to the nature of leucocytosis, based upon a study of the morphology of the leucocyte in the different stages of pneumonia. He believes that leucocytosis may be produced, first, by increased activity of the lymph nodes, as in syphilis and leucæmia. Second, it may be due to failure of devel-

opment of lymphocytes into multinuclear cells. So there may be lymphocytosis without lymphatic hyperplasia. Third, leucocytosis may be due to the failure of further development of polynuclear forms and their consequent accumulation in the blood. In short, leucocytosis, according to Bieganski, is a result of the prolongation of the life history of leucocytes induced by bacterial products circulating in the blood. He calls attention to the sudden appearance of eosinophile cells and of blood plates after the disappearance of multinuclear leucocytes in pneumonia, and cites Zappert (9) and others to support the belief that eosinophile cells are older "fatty" forms of multinuclear leucocytes.

The proof of his theories demands the previous settlement of nearly all the questions now open concerning the leucocyte. That a noxious bacterial poison should exert a preservative influence on the leucocyte it is not natural to suppose. The condition of the blood in leucæmia is not generally regarded as a leucocytosis; while in pseudo-leucæmia there is much lymphatic hyperplasia without leucocytosis. The multinuclear leucocyte, as stated by many authorities, may either break up into blood plates or develop into an eosinophile cell, but it certainly does not develop indifferently into both elements, and it is still doubtful if it is the forerunner of either. According to various observers, the blood plates may be preformed normal elements in the blood (Bizzozero (10)), or broken nuclei of multinuclear leucocytes (Lilienfeld (11)), or globulin particles (Lowit (6)); and the eosinophile cells may be older forms of multinuclear leucocytes (9), or a separate order of cells developed from the eosinophile elements of marrow (H. F. Müller (12), Goldscheider and Jacob (18)); but all these questions are far from solution.

An examination of the foregoing theories reveals in their foundation a very considerable amount of surmise associated with a moderate amount of proof. The incompleteness of our knowledge of chemotaxis; the assumption that all kinds of leucocytes are developmental forms in one series and not representatives of three different series (H. F. Müller (12), Foster (13)); the very capricious behavior of the leucocytes under various physiological as well as

pathological conditions; especially the sudden appearance, in hyperleucocytosis, of enormous numbers of adult multinuclear elements—all these considerations might well raise a doubt as to the validity of our knowledge of leucocytosis. In view of the contradictory evidence, Schulz exclaims that "the whole subject of the existence and meaning of leucocytosis is still '*terra valde incognita*,' and demands extended experimental research." It was apparently this general view of the uncertain foundations of the subject that led Rieder (4) and Schulz (7) to maintain that no sudden increase in the sum total of leucocytes in the blood ever occurred in leucocytosis. As Rieder, at the close of his monograph, states this last view: "There is no definite evidence of an increased outpour of leucocytes from the blood-producing organs, still less of an increase of the same in the blood, nor of an abnormal gathering of wandering cells from the tissues. It must be regarded as much more probable that leucocytosis is not an increase of the sum total of leucocytes circulating in the blood, but only an abnormal distribution of those already existing in favor of the peripheral vessels." Schulz, examining the blood from peripheral and central vessels in dogs, guinea-pigs, and rabbits, was able to convince himself of the correctness of Rieder's hypothesis. During the stage of hyperleucocytosis in peripheral vessels he found no corresponding increase, and even demonstrated a decrease of leucocytes in the central vessels. Whatever may be said of his technique, which will be discussed later, at least an equal number of contrary results are necessary to disprove his position. The present study of the writer was intended to prove, by an independent series of experiments, the truth or falsity of the Rieder-Schulz theory. It was hoped by demonstrating the presence or absence of a change in the sum total of leucocytes in the circulating blood, to settle the question whether the present conception of leucocytosis is based on fact or upon unreliable experimental data.

Technique.—In order to determine the possible changes in the general distribution of leucocytes that may occur after intravenous injection of bacteria, specimens of blood were examined from the central vessels as well as from the peripheral veins, from which the estimation of leucocytosis

is usually made. The microscopical examination of the organs was then demanded, to locate, if possible, the lodging place of the leucocytes found to disappear from the circulating blood after bacterial injections. Rabbits were chosen for the experiments because of the ease with which they are handled, and on account of their comparative freedom from physiological leucocytoses. The experiments of Schulz were repeated a number of times as he performed them, and later an endeavor was made to eradicate some of the features of his technique that were deemed incompatible with accurate results. Accordingly, some of the animals were killed by breaking up the medulla; the abdomen was rapidly opened, and five or six specimens of blood were drawn, by as many "mixers," from the central vessels. This procedure consumed in Schulz's hands usually not longer than fifteen minutes. The writer's specimens of blood were drawn, through a fine needle puncture, in from five to eight minutes after the animal was touched, and no specimen was drawn after the blood pressure became so markedly reduced that the blood failed to spurt from the vein or artery. Several rabbits had to be rejected on this account.

The method used in estimating the leucocytes was the same as employed by the writer in a previous study of the leucocytosis of pneumonia (14), and which, though more laborious, has in his hands given more uniform results than has the acetic acid method. That the acetic-acid method proves extremely unreliable in the experience of others is well shown by some of Schulz's results, in which the estimates of the same vessels varied as much as two hundred and fifty per cent.

The Thoma-Zeiss erythrocytometer was used, the blood diluted a hundred times in three-per-cent. salt solution tinged with gentian violet, and the red cells were thus not dissolved. Changes in the number of red cells and blood plates were therefore regularly noted, and it was possible to detect appearances of the destruction of leucocytes by the media injected. An objection to this method is, of course, the comparatively small number of leucocytes actually counted. This defect was partly remedied either by drawing a double amount of blood into the pipette, making

the dilution 1 to 50, or more frequently by using two counting chambers and counting a double field in each. The double field, though only partly inclosed by the lines of the Zeiss instrument, can be accurately followed without a mechanical stage, by counting over an extra quarter field on each side of the present body of four hundred squares. With a mechanical stage as many leucocytes can readily be counted as in the acetic-acid method.*

After drawing the specimens of blood, the liver, lungs and heart, kidney, spleen, and one femur were removed and hardened for microscopical examination in various agents—such as alcohol, forty to ninety seven per cent; Müller's fluid; Lang's fluid; one per cent. bichloride of mercury; formalin, two, five, and ten per cent. The best preservation was obtained from alcohol, eighty per cent., replaced in twelve hours by ninety-seven per cent. The further procedure with the organs will be described under the report of the microscopical examination.

Dry preparations of the blood were made and stained, with or without heating, by saturated alcoholic solution of eosin and methyl blue or Gage's hæmatoxylin. Of these dry preparations it may be said only that they showed the typical forms described by Okintschitz (15) in normal rabbits' blood, and the great preponderance of uninuclear elements commonly found in hypoleucocytosis.

The order of the experiments was as follows: First, the normal number of leucocytes in the central vessels was determined in rabbits killed by breaking up the medulla, as no sufficient data on this question could be found. Following this, the same vessels were examined, at intervals of five minutes to two hours, after injecting into the middle-ear vein 0·5 to 1·5 cubic centimetres of a three weeks' old broth culture of *Bacillus pyocyaneus*. The injection of this medium was found to reduce the number of leucocytes in the opposite ear vein progressively for at least three hours. Finally, in view of the very great disturbance of the circulation likely to follow destruction of the medulla and immediate failure of respiration, the effect of deep ether narcosis on the blood of the large jugular vein was determined, and all these experiments were repeated on rabbits

* See Eilzholz, *Wien. klin. Woch.*, 1894, No. 32.

thus anæsthetized. To determine the effect of ether narcosis, the rabbit was rapidly bound to a frame and the internal jugular vein exposed by a few short incisions. The specimen of blood was then drawn from the vein and the animal immediately released. By completing this operation within ten minutes, the effects of cooling and exhaustion were practically avoided. At the end of a half hour the same animal was deeply etherized, and specimens were examined from the same vessel.

Effect of Ether on Leucocytes in the Jugular Vein. Leucocytes per Cubic Millimetre.

Number.	Normal.	Fully etherized.
1	6,250 6,500	7,000 7,000
2	7,000 7,000	6,000 7,000
3	6,000 6,250	6,500 7,000

Leucocytes per Cubic Millimetre in Blood of Normal Veins.

I. Examined after Rupture of Medulla.

Ear.	Portal.	Hepatic.	Small mesenteric.	Splenic.	Superior vena cava.	Inferior vena cava.
10,000	10,000	7,500	8,500	.....	7,500	.....
9,800	.....	4,500	9,250	.....	.....	.....
10,500	.....	4,500	12,000	.....	6,000	.....
10,000	9,000	9,000	.....	9,000	6,500	.....
11,000	13,000	13,500	.....	12,000	8,000	.....
12,000	13,000	7,000	.....	13,000	6,000	* 6,800
12,000	.....	10,500	9,000	14,000	7,000	.....
11,500	.....	.....	12,000	13,500	6,000	.....
11,000	.....	10,000	10,500	12,500	6,000	5,500
11,250	.....	12,500	7,000	12,000	5,000	.....

II. Examined under Ether.

						Renal.
8,000	.....	.....	10,000	11,500	5,500	7,000
7,000	.....	.....	9,500	.....	.....	5,000
9,000	.....	9,500	10,500	11,000	5,000	.....
12,000	.....	†7,500	.....	.....	.....	.....
8,000	.....	4,500	.....	.....	.....	.....
6,500	.....	3,500	.....	.....	.....	.....

* Hepatic vein close to vena cava. † Puncture of liver.

Leucocytes per Cubic Millimetre in Blood of Veins after Injection of Bacillus Pyocyaneus.

I. After Rupture of Medulla.

Ear vein, before injections.	Ear vein, after injections.	Hepatic.	Mesenteric.	Splenic.	Superior vena cava.	Inferior vena cava.	Puncture of liver.	Time of examination after injection.
12,000	3,000	3,000	4,000	6,500	2,500	.....	.....	2 hours.
12,500	2,000	1,500	3,000	5,500	1,750	1,500	.....	2 hours.
7,500	3,000	5,500	2,500	5,500	2,250	4,000	.....	2 hours.
10,000	{ 500 / 500	1,000	1,000	2,000	1,250	2,000	.....	2 hours.
10,000	15,000	.....	2,500	.....	2,000	.....	.....	2 hours.

II. Examined under Ether.

						Renal.		
.....	.....	.....	.....	.....	.....	.....	2,500	10 min.
8,000	5,000	.....	3,500	2,500	.....	3,500	3,000	15 min.
10,000	4,500	.....	3,000	2,500	.....	2,250	3,500	15 to 20 min.
8,000	2,500	.....	4,000	8,000	.....	3,250	10,000	30 min.; liver puncture after death.
10,000	5,250	.....	.....	4,500	.....	.....	3,000	30 min.
7,000	2,000	.....	2,000	2,250	1,500	.....	2,500	30 min.
7,000	2,000	.....	2,000	1,000	.....	1,000	1,250	30 min.
7,000	2,500	.....	2,000	1,250	.....	2,000	1,000	1 h. 30 min.
7,500	500	.....	1,000	....	.....	.....	.....	2 h. 20 min.

Leucocytes per Cubic Millimetre in Blood of Normal Arteries

I. After Rupture of Medulla.

Ear vein.	Small mesenteric.	Splenic.	Aorta.		Renal.
			Arch.	Abdominal.	
10,000	3,500	.....	3,000	5,000	....
7,500	.....	.....	2,500	2,500	....

II. Examined under Ether.

12,000	4,500	7,500	4,500	.....	....
8,000	8,500	5,250	4,000	5,000	5,500
8,000	8,000	6,500	3,000	3,500	4,500
6,500	7,000	7,000	.....	.....	9,000

Leucocytes per Cubic Millimetre in Blood of Arteries after Injections.

I. After Rupture of Medulla.

Ear vein, before injection.	Ear vein, after injection.	Small mesenteric.	Splenic.	Aorta, arch.	Aorta, abdominal.	Large mesenteric.	Renal.	Time.
8,500	2,500	2,500	1,500	1,500	2,000	.....	.....	1 hour.
10,000	1,500	1,500	1,750	1,750	1,750	.....	.....	2 hours.

II. Examined under Ether.

8,000	4,500	.....	1,250	.....	.....	1,000	1,500	10 min.
12,500	5,000	2,000	1,500	.....	2,000	.....	1,500	25 min.
10,000	5,000	.....	2,500	.....	.....	2,000	.....	30 min.
7,500	500	2,000	.....	2,000	2,000	500 250	.....	2 h. 20 min.

It will be seen from the preceding tables that during the stage of hypoleucocytosis a uniform diminution of the leucocytes can be demonstrated in all parts of the arterial and venous circulation. Even following, in general, Schulz's own procedure, but drawing the blood from the vessels rather more rapidly than he did, and avoiding much of the change resulting from lowered blood pressure and stagnation of blood in the large veins—factors which apparently disturbed his estimates—this uniform diminution of leucocytes will be still found, and an opposite conclusion to his must be reached. When, however, results obtained in etherized rabbits are compared, this contrary conclusion becomes indisputable. The leucocytes can be found neither in the arteries nor in the veins. Indeed, after a careful examination of Schulz's published tables the writer is quite unable to draw from them any such conclusion as he deduces. Following three different methods, after rupture of the medulla, in chloroform narcosis, and with no anæsthetic, the normal animals he examined are too few to serve as a guide, and his estimates of the leucocytes in the central vessels are so widely divergent that any conclusion as to either increase or decrease is absolutely prohibited.

Destination of the Leucocytes in Hypoleucocytosis.—If, then, the leucocytes disappear, as it seems, from the circulating blood during hypoleucocytosis, one may rightly de-

mand to know what becomes of them; nor is the proof of their disappearance complete until their destination has been established. The earliest clew to the fate of the leucocytes was furnished by Wyssokowitsch (16), when, in 1886, he found that injected bacteria rapidly disappeared from the blood, and were to be found by Gram's stain in the lumen of the capillaries and in the endothelial and fixed connective-tissue cells of the liver, spleen, and kidneys. In a further study of the same subject (17) he located the bacteria at the same points, but claimed that the leucocytes had taken no part in the process of transfer. In 1892 Werigo (18), without regard to the question of the disappearance of leucocytes from the circulation in hypoleucocytosis, found that in the capillaries of the liver, spleen, and kidneys there was a large increase of leucocytes within a few minutes after intravenous injection of bacteria. These leucocytes, or phagocytes, were apparently in the act of transporting bacteria to the endothelial cells of the hepatic capillaries. In many cases the leucocytes were so abundant as to form minute emboli in the capillaries. Similar appearances, less marked, were seen in the spleen and kidney. He also described the normal flat endothelial cells of the hepatic capillaries as much swollen, often partly occluding the lumen of the capillary, sending out protoplasmic processes to entangle the passing leucocytes. One or several leucocytes were sometimes seen completely englobed by the endothelial cells, forming a lenticular giant cell, the nuclei of which were strung along the capillary wall.

With these data in mind, the microscopical examination was made of the organs of the rabbits used in the preceding experiments. The tissues, having been hardened as described, were imbedded in celloidin, and sections were cut of a uniform thickness of one two hundredth of a millimetre, and stained with hæmatoxylon and eosin by Gram's method, and by Loeffler's alkaline methyl blue. As a control, the organs of normal rabbits were first examined. In the normal livers the writer found the capillaries to present all the appearances Werigo describes after the injection of bacteria, except thrombi in the vessels and bacteria in the cells. Many of the endothelial cells were quite flat, their bodies invisible, and their nuclei projecting characteristi-

cally into the lumen of the capillary. Many others, however, were much thicker, occupying a large part of the capillary lumen, containing often pigment granules, apparently sending processes out into the capillaries. All stages of the so-called "giant cells" were seen, from a single well-defined leucocyte adherent to an endothelial cell to a long protoplasmic mass containing a half dozen nuclei. These appearances were too uniform and distinct to be regarded as artifacts, and were seen in every one of nine apparently normal cases examined.

On comparing sections of normal livers with those of the animals killed after injection of bacteria, it was not easy to convince one's self that there was any increase in the number of leucocytes in the capillaries. Only once was such an increase unmistakable, when numerous small thrombi were found. each containing twenty to a hundred polynuclear leucocytes. But such thrombi, having occurred once in twenty cases examined, might reasonably be rejected as evidence of an invariable increase of leucocytes.

The swelling of the endothelial cells seemed rather more marked and more frequent than in the normal livers, but even after two hours many endothelial cells remained quite flat and seemed entirely unaffected by the influences which had caused such great changes in their immediate neighbors. Such features gradually became so striking as to suggest some real difference in character between the swollen and the flat cells. A thorough search over sections of the liver and other organs, stained by Gram's method or by Loeffler's methyl blue, very seldom revealed any bacteria within the leucocytes or endothelial cells. This negative result may be partly explained by the comparatively small quantity of the culture injected. No evidence was found of either mitotic or amitotic division of leucocytes.

A comparison of the general appearance of the liver tissue before and after injection failing to give satisfactory evidence of a uniform increase of leucocytes, it was decided to make a count of their numbers in the cubic millimetre of hardened tissue. For this purpose a mechanical stage and a Zeiss one-twelfth immersion lens were used, and the leucocytes in one square millimetre of each section were counted. The sections being one two-hundredth

millimetre in thickness, the result of the count was multiplied by two hundred, which gave the number of leucocytes in one cubic millimetre of tissue. An attempt was made to count the leucocytes in the small arteries and veins before and after injections, but the results were so nearly equal in the two cases that no conclusions were possible.

An examination of the subjoined tables will show that, according to this method of estimate, there was a steady increase of leucocytes in the hepatic capillaries for at least two hours after the injection of *Bacillus pyocyaneus* into the ear vein. During the same period a marked diminution of leucocytes had been demonstrated in the circulating blood. In four instances two cubic centimetres of the sediment from a three-days-old broth culture of *Bacillus anthracis* was injected, instead of the smaller quantity of *Bacillus pyocyaneus*.

Leucocytes per Cubic Millimetre of Liver Tissue.

I. Normal Livers.

Ear vein, before injections.	Ear vein, after injections.	Leucocytes per cubic millimetre.	Injection.	Time of examination after injection.
7,500	.....	23,000	.....	.....
12,000	.....	12,500	.....	.....
8,000	.....	20,000	.....	.....
8,000	.....	16,000	.....	.....
6,500	.....	20,000	.....	.....
7,000	.....	10,200	.....	.....
8,500	.....	12,600	.....	.....
8,000	.....	19,600	.....	....
11,000	.....	22,800	.....	.....

II. After Injections.

.....	.....	33,500	Anthrax.	2 min.
.....	.....	35,000	"	6 min.
8,000	4,500	38,000	Pyocyaneus.	7 min.; thrombi.
8,000	4,500	34,000	"	10 min.
.....	.....	42,500	Anthrax.	20 min.
8,000	5,000	29,400	Pyocyaneus.	15 min.
12,500	5,000	26,600	"	25 min.
10,000	4,500	34,000	"	30 min.
7,000	2,000	35,000	"	30 min.
8,000	2,500	37,000	"	30 min.
10,000	5,000	51,000	"	30 min.
7,000	2,500	51,500	"	1 h. 30 min.
8,500	3,000	58,000	"	1 h. 30 min.
12,000	3,000	57,000	"	2 hours.
12,500	2,000	55,000	"	2 hours.
7,500	3,000	41,000	.....	2 hours.

The examination of the lungs was made in the majority of cases without previous inflation, as it seemed probable that such treatment would disturb the condition of the capillaries. From a general survey of sections from the lungs prepared in this way, it appeared probable that the pulmonary capillaries contained many more leucocytes after the injections, but any attempt at a numerical comparison was quite impossible. The lungs in the remaining cases were therefore slowly distended with forty-per-cent. alcohol to rather less than their normal size in expiration, and from sections of the organs thus prepared the number of leucocytes in the cubic millimetre was computed as described for the liver. The separation of the nuclei of leucocytes from those of other cells in the pulmonary parenchyma was often difficult and sometimes impossible, but the total error from this source is believed not to seriously affect the comparisons. The injections of *Bacillus anthracis* affected the lungs more powerfully than did *Bacillus pyocyaneus*, the former producing many minute thrombi in the distended capillaries. In the capillary endothelium no changes could be discerned such as were constantly found in the liver.

Leucocytes per Cubic Millimetre of Lung Tissue.

I. Normal Lungs.

Ear vein, before injections.	Ear vein, after injections.	Leucocytes per cubic millimetre.	Injection.	Time of examination.
7,000	.....	40,600	.....	.....
8,000	.....	42,000	.....	.....
8,000	.....	37,800	.....	.....
11,000	.....	41,000	.....	.....

II. Lungs after Injection.

.....	.....	98,000	Anthrax.	6 min.; thrombi.
.....	.....	111,400	"	20 min.; thrombi.
8,000	5,000	42,000	Pyocyaneus.	15 min.
12,500	5,000	85,000	"	25 min.

In the kidney no changes could be discovered in the endothelial or fixed connective-tissue cells, capillary vessels, Malpighian tufts, or larger arteries or veins. The multinuclear leucocytes were counted in twenty Malpighian tufts in

each of five normal kidneys and in five cases after injections. Only multinuclear leucocytes were here counted, because of a difficulty experienced in distinguishing the nuclei of lymphocytes from the short spherical nuclei of the endothelial cells.

Multinuclear Leucocytes in Malpighian Tufts of Kidneys.

I. Normal Kidneys.

Ear vein, before injections.	Ear vein, after injections.	Leucocytes in twenty tufts.	Injection.	Time of examination.
8,000	.....	28	.....	.....
6,500	.....	27	.....	.....
8,000	.....	36	.....	.....
7,000	.....	24	.....	.....
7,500	.....	26	.....	.....

II. After Injections.

8,000	4,500	18	Pyocyaneus.	10 min.
8,000	5,000	12	"	15 min.
12,500	5,000	18	"	20 min.
.....	.....	26	Anthrax.	20 min.
7,000	2,500	30	Pyocyaneus.	90 min.

In the marrow of the femur the leucocytes in the capillaries were often slightly increased in numbers, and rather more of the large uninuclear elements of the marrow appeared to be free in the circulation, but the extent and constancy of these changes were not very manifest. The examination of the spleen did not give convincing evidence either for or against an increase of leucocytes, although pulp cells, endothelial cells, and sections of blood in vessels were carefully compared. The anæmic condition of the spleen found after the injection of bacteria must stand as strong presumptive evidence against any immediate increase of leucocytes in this organ.

Leucocytolysis.—There remains the question of an actual destruction of leucocytes in the circulating blood by bacterial products, and of its importance in accounting for the disappearance of these cells in hypoleucocytosis. Most investigators have failed to find in the blood definite traces of the solution of leucocytes. The weight of opinion is against the belief that the blood plates are products of the disintegration of multinuclear leucocytes. It is possible,

however, that among the multitude of irregularly defined granules constantly met with in the routine of blood examinations some may be products of the disintegration of leucocytes. Among the authorities who maintain that actual destruction of leucocytes is a factor in their disappearance in hypoleucocytosis may be mentioned Lowit, Roemer, S. S. Botkin, Holtzmann, E. Botkin, *et al.* (19). That no such destruction occurs in the blood is more or less strictly held by Schulz, Rieder, Werigo, Medwedeff, Goldscheider and Jacob (19). Since the publication of E. Botkin's (19) experiments on the solubility of leucocytes in peptone there remains little doubt that injection of bacterial products directly into the circulation may destroy a considerable number of leucocytes.

Botkin treated pus with solutions of peptone, and was able to follow, under the microscope, the changes that occurred in the leucocytes. Within fifteen minutes after treatment with peptone the granules began to disappear from the protoplasm of the leucocytes; they became translucent, finally invisible, while the nuclei lost their color and gradually fell in pieces. In twenty-four hours eighty per cent. of the leucocytes in the mixture had disappeared.

Throughout the present work, after the injections, granular particles were constantly found in the blood, which might equally well have been regarded as deformed blood plates, or as fragments of the nuclei of leucocytes. These granules were more abundant if the blood was allowed to stand in the mixer for an hour. In such specimens fragmentation and complete solution of the protoplasm of leucocytes could be plainly followed, while the nuclei, more difficult of solution, were seen to break up into irregular, faintly stained granules. At the end of two hours a diminution in the number of leucocytes was regularly noted. After four hours this diminution became marked, and in some specimens, examined eighteen hours after drawing, it was impossible to find a single leucocyte among the clumps of developing bacilli.

No such changes occurred in the blood drawn from healthy rabbits before injection.

If the injections of one cubic centimetre of a broth culture of *Bacillus pyocyaneus* can in one hour so affect the

blood that the solution of leucocytes can be discerned in the counting chamber, it is probable that the larger injections used by most experimenters have furthered the impoverishment of the blood by direct leucocytolysis. It seems probable also, from analogy, that the bacterial products thrown into the circulation at the onset of an acute disease like pneumonia must destroy a moderate number of leucocytes. But, since nearly complete disappearance of leucocytes from the blood can be produced by large injections within a few minutes, one must at present be content to regard leucocytolysis as only an accident in the course of hypoleucocytosis.

Summary.—1. Within eight minutes after rupture of the medulla in rabbits very little change occurs in the number of leucocytes in the blood of the central vessels.

2. Ether narcosis in rabbits has very little effect on the location of leucocytes in the circulating blood.

3. The view of Rieder and Schulz, that no change in the sum total of leucocytes in the blood ever occurs in leucocytosis, is incorrect, and may be disproved by examination of rabbits' blood in the stage of hypoleucocytosis, either after rupture of the medulla or, more conclusively, in ether narcosis.

4. After intravenous injection of certain bacteria and their products, the majority of the leucocytes, especially the multinuclear forms, disappear uniformly from all parts of the arterial and venous circulation.

5. The leucocytes that disappear after bacterial injections are to be found, more or less stationary, in the capillary vessels, especially in the lungs and liver.

6. The appearance of the endothelial cells of the hepatic capillaries indicates that these cells may take more than a passive part in detaining the leucocytes within that organ.

7. Leucocytolysis is apparently a secondary and unessential factor in the production of hypoleucocytosis.

8. It remains an open question whether hypoleucocytosis depends upon a simple mechanical sifting of swollen and cohesive leucocytes by the capillary endothelium or upon a determination of these leucocytes, by chemotactic influence, to specialized capillary endothelium in the viscera.

9. The appearance of the hepatic capillary endothelial

cells, both before and after the injection of bacteria, points to a possible function of the liver as the physiological scavenger of the body, and, in pathological conditions, as a special organ of phagocytosis.

Addendum.—While the results of the present work were being prepared for publication, the extensive and valuable study of Goldscheider and Jacob (20) came under observation. Their experiments were specially suggested by the theories of Lowit and Schulz.

By injecting minute doses of a glycerin extract of the spleen they succeeded in producing marked increase in leucocytes without appreciable previous decrease.

They call attention to the remarkable variations in the numbers of leucocytes found by Schulz in the central veins, and discredit all his results because the specimens of blood were drawn after rupture of the medulla or other vitiating procedures. In two experiments they find the leucocytes diminish greatly in the ear vein immediately after rupture of the medulla, and infer, but do not attempt to prove, that there is a corresponding and simultaneous change in the central vessels. After convincing themselves that ether narcosis has no effect on the location of leucocytes, they examine the blood of three normal etherized rabbits in the stage of hypoleucocytosis and one in hyperleucocytosis, and thus attempt to combat the more numerous experiments of Schulz.

If Goldscheider and Jacob had proved that rupture of the medulla caused as pronounced and immediate a change in the central veins as it does in the ear vein, they might justly discredit Schulz's results. But the writer's experiments show that, if the operation is done within four to eight minutes, and before the blood pressure is markedly reduced, blood may be drawn from the central vessels before any appreciable change occurs in the number of leucocytes. Many of Schulz's specimens were taken within this time, and some of his operations were performed under chloroform. As to the actual data presented, the weight of evidence must still remain with Schulz.

One of the most valuable features of Goldscheider and Jacob's work is to be found in their report of the microscopical examination of the organs, by which they locate,

principally in the pulmonary capillaries, the leucocytes that disappear after injections of bacteria. With one prominent exception, they failed to find in the liver the appearances described by Werigo, and conclude that this organ is little concerned in the phenomena of hypoleucocytosis.

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